By the mid 1930’s, yeast was already known as an important tool in biochemical research. Its most important role in basic science and research was yet to be realized, however, until the mid 1940’s when Dr.’s Winge and Roberts realized that basic Mendelian inheritance could be followed in this organism. For instance, when they followed enzymatic markers in spores from diploid cells (see below), they observed the classic 2:2 segregation. This was considered the rule until 1949 when Dr. Lindegren observed departures from this segregation of markers that occurred too frequently to be ignored. For instance, he occasionally recorded segregation of markers at a 4:0, 3:1, 1:3, and 0:4 in the spores formed from a diploid cell. He coined a term known as “gene conversion” after Winkler’s (1930) theory of recombination bearing the same name and based on similar data seen in other organisms and plant life studied. Simply stated, gene conversion was an observation that two phenotypic characteristics did not always segregate evenly (or even at all) among offspring. Early scientists determined this was due to the “conversion” of one gene into another gene by a yet to be discovered process. Subsequently, it was found that these conversions always occurred near crossover points on chromosomes, and the analysis of gene conversion has played a crucial role in the understanding of the molecular mechanisms of recombination. These discoveries were part of the birth of modern genetics that tied the traits of many life forms studied with the newly discovered but as of yet poorly understood component of all cells, DNA. Yeast’s role in the final determination that DNA carried biological traits was now firmly in place.

Life Cycle

Yeast is somewhat unique as a life form in that it can survive as either a haploid cell, which contains only one set of the 17 chromosomes (1N), or as a diploid cell which contains two sets of the 17 chromosomes (2N) (Fig. 1). The haploid cell therefore carries only 1 copy of each gene it requires to survive, not unlike a bacterial cell. This haploid cell produces offspring through a process called budding in which a new yeast cells slowly “buds” off the parent cell until it reaches an appropriate size when it finally tears off the “mother” cell. This budding process in haploid yeast utilizes mitosis as its’ sole source of reproduction (as opposed to meiosis) and happens quickly as a normal S. cerevisiae haploid cell divides every 40 minutes or so.
Figure 1. Life cycle of yeast.

Diploid cells are formed when two haploid cells "mate." This mating process is somewhat complicated and has amazing similarities to the attraction characteristics of many animals, i.e. the use of pheromones. Briefly, haploid yeast cells come in two types: Mat $a$ (mating type $a$) and Mat $\alpha$ (mating type $\alpha$). A Mat $a$ haploid yeast cell produces the mating pheromone “a” which it secretes outside the cell. This Mat $a$ pheromone is detected by another haploid yeast cell that is the opposite mating type, i.e. a Mat $\alpha$ cell. This also happens in the opposite direction with a Mat $\alpha$ cell producing its pheromone that is detected by a Mat $a$ haploid cell. Importantly, two yeast of the same mating type cannot mate!!! If these two different cell types are close enough to touch, they will fuse to form a single cell that contains the complete chromosomes of each of the original cells, hence the 2N chromosome count. These cells now contain 2 copies of each gene the yeast cell requires to survive. These diploid cells can now divide either by budding (mitotic reproduction) as describe above or, under harsh conditions, will form spores containing 4 new haploid yeast (2 Mat $a$: 2 Mat $\alpha$). This spore forming reproduction in yeast utilizes meiosis to form the 4 haploid spores from one diploid yeast cell (1 2N cell to 4 1N cells). These spores will break and release the new haploid yeast when conditions are favorable for growth.
Requirements for growth

Very similar to bacteria (prokaryotes) that you have previously used in other labs, yeast (eukaryotes) cells have similar nutritional requirements and optimal temperatures for growth. Yeast can be grown on solid agar media on a petri dish, or in liquid cultures in a shaking water bath. Yeast have a much wider temperature range for growth than most bacteria, however. They have the ability to grow at temperatures as low as 12°C or as high as 42°C.

Nutritional requirements for yeast have been clearly defined over the years and the actual pathways for both utilization and synthesis of these requirements have been discovered. For instance, the pathway for Uracil production (a metabolic precursor) is required in yeast for survival. If any biochemical step in this pathway is destroyed by a DNA mutation, the yeast will die unless uracil is added to the growth medium. A yeast cell that has lost the ability to synthesize uracil due to a DNA mutation somewhere in the biosynthetic pathway for uracil is called an auxotroph. These uracil auxotrophs must be supplied with uracil in the growth media or they will die. A normal (called wild type, WT) yeast cell that can make uracil is called a prototroph. These cells do not require uracil in the media as they can synthesize it on their own. There are many of these biosynthetic pathways know in yeast, with auxotrophs identified in each of them. It is this characteristic of yeast (auxotroph/prototroph) that made this organism one of the most powerful genetic tools of the 20th century. A scientist can follow hundreds of genetic markers simply by changing what he adds to the growth media and asking whether the yeast live or die!!! We will be utilizing the characteristics of two auxotrophs in the course of our yeast work. One yeast auxotroph we will use is the ura gene described above. These yeast strains must be grown with uracil in the media as the cells lack the ability to produce it due to a mutation in the ura metabolic pathway. The second yeast auxotroph we will use is the ade gene whose metabolic pathway is described below.

EXPERIMENTS

WEEK 1

The purpose of this week’s experiment is to observe and analyze gene complementation in yeast as well as determine the mating types of experimental yeast haploid strains.

Your experiment this week is to determine what specific genes, if any, are mutant in the adenine biosynthetic pathway in several unknown haploid yeast strains. You will receive four unknown yeast strains, each of which may or may not contain a mutation in the adenine biosynthetic pathway (below). Therefore, strains that are mutant in this pathway are adenine auxotrophs (ade-) i.e. they require the addition of adenine to the growth media to survive. In
addition these four haploid yeast strains are not all the same mating type, they could be either Mat a or Mat α. Remember, yeast of the same mating type will NOT mate and form diploids.

You will determine:

1) at what stage each of four unknown yeast strains are blocked in the ade pathway, if at all.

2) what mating type each strain is based solely on the characteristics of the strains AFTER you perform a mating experiment.

**Adenine Biosynthetic Pathway:**

Yeast test crosses are useful in yeast genetics because unknown mutations can be identified after haploid yeast mate to form diploids. For instance, a haploid yeast strain that has an *ade2* mutation, which disrupts the adenine biosynthetic pathway, cannot grow on media lacking adenine (*ade* auxotroph). If you mate this haploid to another *ade* auxotroph, which is an *ade5* mutant, the resulting diploid strain will be an *ade* prototroph because it will contain wild type copies of both the *ade2* gene (from the *ade5* mutant strain) and the *ade5* gene (from the *ade2* mutant strain) and the adenine biosynthetic pathway will be complete (Fig. 2). In contrast, if you had mated the *ade2* mutant to another *ade* auxotroph, which was also an *ade2* mutant, the resulting diploid would not be an *ade* prototroph because the pathway would still be blocked.

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**Figure 2. Adenine Biosynthetic Pathway.**
The production of a wild-type phenotype when two different mutations are combined in a diploid is called “gene complementation”. The wild type copies of the genes now complement each other and the biochemical pathway is complete (Fig. 3).

**Complementation testing**

<table>
<thead>
<tr>
<th>1. Complementation</th>
<th>2. No complementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal chromosome</td>
<td></td>
</tr>
<tr>
<td>Defective gene</td>
<td>Functional gene</td>
</tr>
<tr>
<td>Parental chromosome</td>
<td></td>
</tr>
<tr>
<td>Functional gene</td>
<td>Defective gene</td>
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</tbody>
</table>

**Conclusion:** \( m_1 \) and \( m_2 \) are in different genes. \( m_1/m_2 \) has wildtype phenotype because one chromosome supplies gene \( G \) function while the other supplies gene \( R \)

**Conclusion:** \( m_1 \) and \( m_2 \) are in the same gene. \( m_1/m_2 \) has mutant phenotype because organism has no gene \( G \) function.

**Figure 3. Gene complementation.**

**Procedures**

Obtain 1 master plate containing yeast strains

CTY2c

CTY4c

CTY415

CTY416

**Mating Yeast:** Obtain 1 YPD agar plate/group of 2 and section off into 3 sectors by marking on the bottom of the plate with a marker (see below, left). These YPD plates are complete media plates and provide yeast with all nutrients they require regardless of any mutations in biosynthetic pathways. Use a sterile toothpick to pick a SMALL amount of strain CTY2c from the master plate and smear it into a circle about the size of a dime onto the YPD plate. BE GENTLE, do not jab the toothpick into the agar. Next, use a new toothpick to pick a small amount of strain CTY4c and smear it directly into the smear you made with CTY2c. By “mixing” the two strains together into a single patch, the yeast can now mate. Repeat this procedure for strain CTY4c mixed with strain CTY415 on a different section of the YPD plate. Repeat procedure again by mixing strain CTY4c with strain CTY416 in the third section of the YPD plate. **Save the master plates.** When you are done, you will have three patches on the plate, each representing a test cross. Incubate these plates overnight at 30°C.
The next day, obtain 3 new YPD plates/group and sector each one into thirds. Using a sterile toothpick, obtain a SMALL amount of yeast from one of the mating patches and streak onto one sector of your new YPD plate. Repeat this procedure (using new toothpicks each time) to streak the other two sectors on the plate (Fig. 4). These three streaks will help insure you obtain individual colonies. **Make sure you label this plate to indicate which specific mating it was made from.** Repeat this procedure with the other 2 test crosses until you have 3 YPD plates, each representing a single test cross. Incubate these plates at 30°C for two days.

![Fig. 4. Plating and streaking yeast.](image)

After 2 days of growth, you should see isolated colonies within the streaks. **Indicate the colors of the colonies in these streaks below.** Have the colors changed from the original test strains? **Save these plates.** Obtain 4 -Ade plates. On three of the plates, sector into fourths (see below) and label according to the test crosses you performed (i.e. one YPD plate label “CTY2c X CTY4c. A second YPD plate label “CTY4c X CTY415 and so on.). These plates lack adenine in the media so any yeast that grow on them must provide their own adenine via the pathway described above. You will now streak individual colonies onto this -Ade plate from the test cross YPD streak plates. For instance, pick 4 individual colonies from the YPD streak plate of mating CTY2c X CTY4c and streak onto your labeled –Ade plate; one streak per section. Repeat this procedure with the other two YPD streak plates. **Make sure you streak 3 white colonies and 1 red colonies from each plate UNLESS you have only colonies of one color, i.e. red only.** On the fourth –Ade plate, streak the original test cross strains from the master plates leftover from your lab day. You should now have 4 –Ade plates, 3 sectioned into fourths with streaks of
colonies of each test cross in the appropriate section, the fourth plate should have one streak of each of the 4 unknown original yeast strains (Fig. 5). Incubate these plates at 30°C for two days.

Fig. 5. Streaking yeast.

Score each cross for growth or no growth and record your results in the section below. Save these plates and bring them to class.

Answer these questions in the sections below for each mating.

What do you notice about the color of the colonies and their ability to grow on –Ade plates?

What is the possible mating type of each of the test strains (Mat a or Mat α)? Explain.
Which one of the three test crosses represents a cross that ultimately results in a WT diploid yeast strain?

Explain all your answers and identify any problems that occurred.

1) CTY2c X CTY4c

2) CTY4c X CTY415

3) CTY4c X CTY416